Supplemental Online Content

Gottlieb RL, Nirula A, Chen P, et al. Effect of bamlanivimab as monotherapy or in combination with etesevimab on viral load in patients with mild to moderate COVID-19: a randomized clinical trial. *JAMA*. doi:10.1001/jama.2021.0202

This supplemental material has been provided by the authors to give readers additional information about their work.

eTable 1. Pseudovirus neutralization of spike variants in the presence of bamlanivimab and etesevimab

Spike Variant	Bamlanivimab		Etesevimab	
	IC_{50} µg/mL (95% CI)	**Fold-shift in IC_{50}**	**IC_{50} µg/mL (95% CI)**	**Fold-shift in neutralization**
Wuhan[a]	0.01 (0.01, 0.02)	1	0.13 (0.07, 0.41)	1
E484K	>1	>100	0.57 (0.36, 1.11)	4.4
E484Q	>1	>100	0.17 (0.10, 0.36)	1.4
F490S	>1	>100	0.1 (0.04, >2)[b]	0.8
S494P	>1	>100	0.07 (0.03, >2)[b]	0.5

Abbreviations: CI, confidence interval; E, glutamate; F, phenylalanine; IC_{50}, concentration inhibiting maximal activity by 50%; K, lysine; P, proline; Q, glutamine; S, serine; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.
Note: IC50 and IC90 values were calculated as absolute IC values.
[a]SARS-CoV-2 S Genbank MN908947.3.
[b]Confidence interval cannot be calculated.

eTable 2. Outcomes for post hoc and exploratory analyses

	Bamlanivimab 700mg	Bamlanivimab 2800mg	Bamlanivimab 7000mg	Bamlanivimab + Etesevimab	Placebo
***Post Hoc* Analyses**					
COVID-Related Hospitalization or Emergency Room Events at Day 29 in high-risk patients[a]					
Number of patients, N	37	30	34	31	52
Events, (%)	1 (2.7)	1 (3.3)	2 (5.9)	0 (0.0)	7 (13.5)
vs Placebo, difference (95% CI)	-10.8 (-21.4, -0.1)	-10.1 (-21.4, 1.2)	-7.6 (-19.8, 4.6)	-13.5 (-22.7, -4.2)	
P value	0.13	0.25	0.31	0.042	
Exploratory Outcomes					
Virological Measures					
SARS-CoV-2 Viral Load Change from Baseline at Day 3					
Number of patients, n/N	96/101	98/107	93/101	96/109	141/152
Viral load at Day 3, mean (SD)	5.00 (1.79)	4.71 (2.31)	5.02 (1.77)	4.83 (2.08)	5.24 (2.55)
Viral load change from baseline at Day 3 vs Placebo, difference (95% CI)	-0.23 (-0.67, 0.20)	-0.43 (-0.87, -0.00)	-0.31 (-0.75, 0.13)	-0.55 (-0.99, -0.11)	
P value	0.30	0.05	0.17	0.013	
SARS-CoV-2 Viral Load Change from Baseline at Day 7					
Number of patients, n/N	98/101	101/107	95/101	95/109	142/152

Viral load at Day 7, mean (SD)	3.42 (1.81)	3.23 (1.81)	3.45 (1.94)	2.80 (1.81)	3.74 (2.07)
Viral load change from baseline at Day 7 vs Placebo, difference (95% CI)	-0.26 (-0.70, 0.19)	-0.40 (-0.84, 0.04)	-0.29 (-0.74, 0.15)	-1.12 (-1.57, -0.67)	
P value	0.26	0.07	0.20	<0.001	
SARS-CoV-2 Viral Load Change from Baseline AUC (Days 0-11)					
Number of patients, n/N	91/101	99/107	90/101	99/109	132/152
Viral Load AUC (Days 0-11) AUC, mean (SD)	42.93 (14.55)	39.82 (17.34)	42.22 (15.95)	38.14 (16.01)	42.84 (19.51)
vs Placebo, difference (95% CI)	-0.50 (-3.82 2.82)	-2.39 (-5.63, 0.85)	-1.67 (-5.00, 1.67)	-6.51 (-9.76, -3.27)	
P value	0.77	0.15	0.33	<0.001	
Clinical Measures					
Total Symptom Score AUC (Days 0-11)					
Number of patients, n/N	80/101	74/107	71/101	80/109	99/152
Total Symptom Score AUC (Days 0-11), mean (SD)	23.54 (20.42)	27.43 (24.54)	29.39 (28.16)	23.40 (18.41)	34.32 (25.59)
vs Placebo, difference (95% CI)	-8.28 (-14.04, -2.53)	-6.59 (-12.46, -0.72)	-8.09 (-14.05, -2.13)	-8.63 (-14.39, -2.88)	
P value	0.005	0.028	0.008	0.003	
Total Symptom Score AUC (Days 0-29)					
Number of patients, n/N	65/101	59/107	54/101	77/109	84/152
Total Symptom Score AUC (Days 0-29), mean (SD)	38.52 (47.77)	39.86 (42.04)	46.49 (58.37)	40.35 (57.11)	50.19 (44.84)
vs Placebo, difference (95% CI)	-6.95 (-21.44, 7.54)	-11.80 (-26.67, 3.07)	-5.27 (-20.54, 9.99)	-5.95 (-19.78, 7.88)	
P value	0.35	0.12	0.50	0.40	

Viral Resistance Measures					
Patients with Treatment-Emergent Bamlanivimab Resistance Variants[b]					
P value	0.26	0.07	0.20	<0.001	
Number of patients, N	98	102	97	102	145
Any occurrence; n, (%)	7 (7.1)	10 (9.8)	11 (11.3)	1 (1.0)	7 (4.8)
Multiple occurrences; n, (%)	4 (4.1)	6 (5.9)	7 (7.2)	0 (0.0)	0 (0.0)

N, number of patients in the analysis population; n, number of patients in the specified category.

[a] ≥65 years of age or with BMI ≥35.

[b] Treatment emergent variants were determined by comparing the sequencing results from the study participant's baseline sample to those obtained from that participant post-treatment. Analysis was then focused on variants that were phenotypically confirmed to be resistant to bamlanivimab (E484K; E484Q; F490S and S494P) and occurred a ≥15% variant allele frequency.

eMethods

Pseudovirus Assay

Spike mutant expression vectors were constructed and used to produce pseudovirus using the delta-G-luciferase recombinant Vesicular Stomatitis Virus system developed by KeraFast. Infectious titers and particle (genome) titers were determined by anti-luciferase FACS and qRT-PCR, respectively; titers for all variant pseudoviral preps fell within 5-fold of that for the parental Wuhan spike virus prep (Genbank MN908947.3). For neutralization assays, viral prep volumes were normalized to equivalent signal output (relative light units), as determined by luciferase activity of cells following infection with serially diluted virus. For the neutralization assay, each antibody was titrated and pre-incubated with a fixed amount of virus for 20 minutes at 37°C. Following pre-incubation, virus:antibody mixtures were added to Vero E6 cells in 96-well plates and incubated for 16 to 20 hours at 37°C. Relative viral infectivity was determined by luciferase assay.

Quantitation of RT-PCR Results

For quantitative endpoints in the trial, nasopharyngeal epithelial "viral load" was derived using the Ct values for the N1 target. If a clinical sample was positive per the diagnostic criteria with only N2, and no Ct determined for N1, the N2 Ct value was used instead. The (log base 10) viral load was calculated from the Ct value according to the following formula:

Log viral load = $(45\text{-Ct})/\log_2 10$

For the purposes of this calculation, qualitatively negative RT-PCR results for SARS-CoV-2 were assigned a Ct value of 45 for N1.

Two Ct values were provided on 2 different genes: N1 and N2. N1 was used as the primary measure; N2 was only used when the Ct value for N1 is not available.

For defining PHVL (persistently high viral load), for any sample with a positive CoV-2 test result, an additional normalization step was taken to reduce pre-analytical variability in the viral load measurements. The cut-off for PHVL was determined by a recursive partitioning approach. The N1 Ct value for any positive sample in these treatment arms (or N2 Ct value if N1 was not available as

previously described) was subtracted by the Ct value for the RNAse P (RP) mRNA target for that sample minus 26.17:

N1/N2 Ct value for that sample – [(corresponding RP Ct value for that sample) – 26.17)] = Normalized N1/N2 Ct value

The correction factor of 26.17 is a historical average value of RP Ct for this assay and was used here to normalize N1/N2 "viral load" calculations to RP.

Following this correction, the RP-normalized log "viral load" was then calculated as follows:

Log viral load = (45 - Normalized N1/N2 Ct value)/ $\log_2 10$

Resistance Variants

Nucleic acid was extracted from 200 µL viral transport media using MagMAX Viral/Pathogen Binding Beads (Thermo Fisher) on a Microlab STAR (Hamilton). The Ion AmpliSeq SARS-CoV-2 Research Panel (Coronavirus_v2pt2) was used for NGS library preparation on the Ion Chef system (Thermo Fisher). It comprised 2 pools of overlapping amplicons ranging from 125 to 275 base pairs and covered >99% of the SARS-CoV-2 genome. A series of experiments was performed to determine optimal nucleic acid input amount and the optimal number of amplification cycles required for each specimen based on cycle threshold (Ct) values from quantitative (q) RT-PCR targeting the nucleoprotein N gene. Templating was performed using Ion 540 chips, and then chips were sequenced on the Ion Torrent S5XL (Thermo Fisher). Reads were aligned to the "hg19_human_trxome_and_coronavirus.fa" reference genome (which included MT019532.1 BetaCoV/Wuhan/IPBCAMS-WH-04/2019) using bwa-mem version 0.7.12 with default parameters. Variants were called using FreeBayes version 1.3.1 with the following parameters: -F 0 -p 1 -K -C 0 -n 5 -w --min-alternate-count 0 --min-alternate-fraction 0. In this exploratory analysis, variants were selected for inclusion if they were annotated as either a missense mutation or an inframe insertion/deletion, had ≥5 reads of variant allele, a minor allele fraction ≥0.1, and a variant allele fraction ≥0.15. Data are reported for known phenotypically confirmed bamlanivimab resistant variants in the spike protein (E484K; E484Q; F490S and S494P) that were identified in clinical samples. Briefly, bamlanivimab resistant variants were determined

through a number of preclinical assays including binding assessment of the antibody to variant spike protein and serial passage studies with live SARS-CoV-2 and subsequent sequencing. Resistance to bamlanivimab was confirmed in pseudovirus assays with specific variants incorporated.

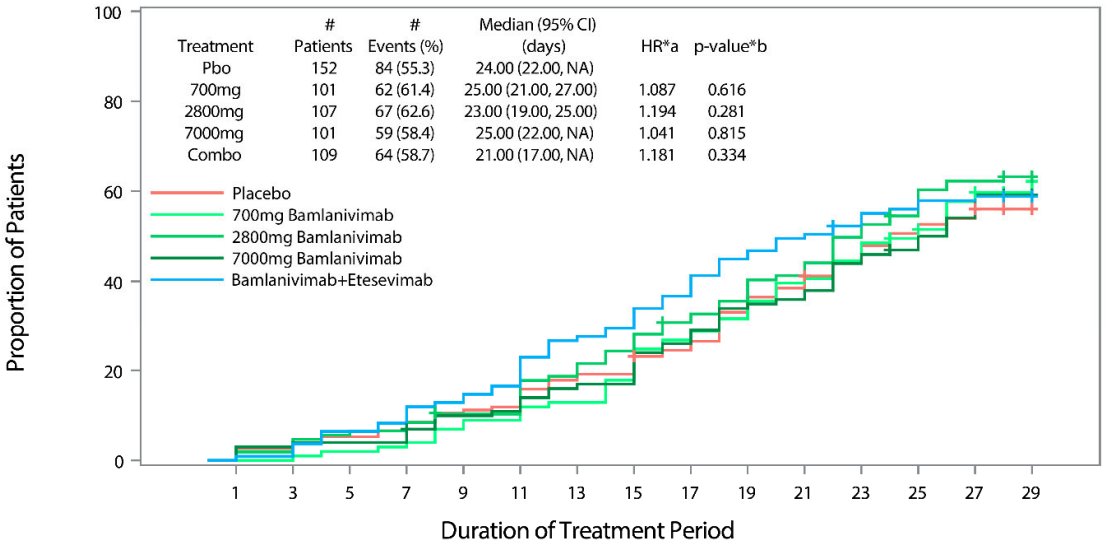
eResults

Additional Post Hoc Analyses

There was an association across placebo and treatment arms between slower viral clearance and hospitalization events (eFigure 4), with a significant correlation between viral load at Day 3 (p=0.0053, Wilcoxon rank-sum test) and Day 7 hospitalization (p=0.00011), respectively. A cut-point analysis was conducted to define a threshold for persistently high viral load (PHVL) to correlate with a hospitalization outcome. Analysis of the full cohort suggested a Ct value of <27.5 (SARS-CoV-2 N1 primer, equivalent to 570,000 NAAT/mL using the FDA SARS-CoV-2 Reference Panel, or log viral load ≥5.27) as a meaningful definition of PHVL on Day 7 which was correlated with symptom severity and hospitalization. The average total symptom score from Day 7 to Day 10 is higher (p<0.001, Wilcoxon rank sum test) in patients with PHVL (mean=3.41) than those without PHVL (mean=1.73). The rate of hospitalizations in patients with PHVL was 10.9% (7/64) versus 0.8% (4/479) in the non-PHVL group (odds ratio 14.6).

The cut-point was derived from a subset of placebo and monotherapy cohort data set at Day 7, so we evaluated the fraction of patients with PHVL across treatment groups, including a prospective evaluation of the combination therapy group. For the placebo group, 20.7% (30/145) of patients had PHVL. In comparison to placebo, a significantly lower number of patients met this threshold in the monotherapy dose arms (700mg: 12.1%, 12/99; p=0.9, 2,800mg: 8.9%, 9/101; p=0.013, 7,000mg: 10.1%, 10/99; p=0.034) and combination (3%, 3/100, p<0.001) treatment groups.

eFigure 1. Time to SARS-CoV-2 clearance

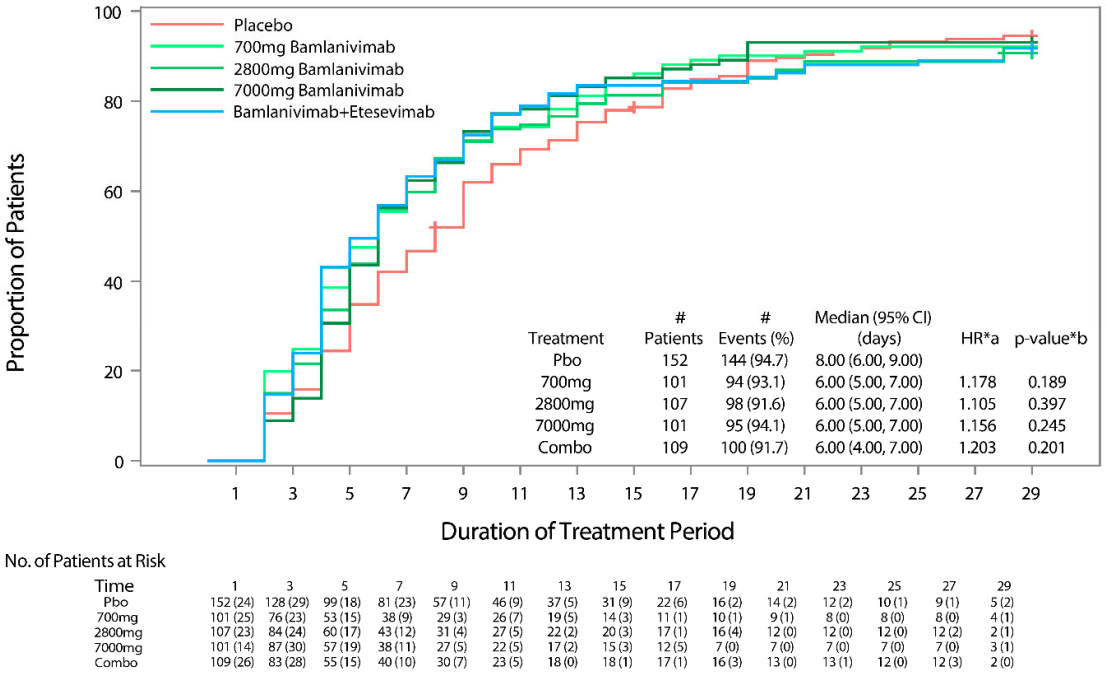


No. of Patients at Risk

Time	1	3	5	7	9	11	13	15	17	19	21	23	25	27	29
Pbo	148 (2)	146 (2)	144 (5)	139 (4)	134 (7)	127 (5)	122 (6)	115 (5)	110 (15)	95 (7)	87 (10)	77 (7)	70 (5)	64 (0)	20 (0)
700mg	101 (1)	100 (1)	99 (2)	97 (5)	92 (3)	89 (1)	88 (12)	76 (4)	72 (7)	65 (5)	60 (8)	52 (3)	47 (8)	38 (2)	10 (0)
2800mg	105 (3)	102 (2)	100 (2)	98 (2)	96 (8)	88 (4)	84 (7)	77 (5)	71 (8)	63 (4)	59 (9)	50 (8)	41 (2)	39 (1)	16 (0)
7000mg	98 (1)	97 (0)	97 (3)	93 (3)	90 (4)	86 (3)	83 (7)	76 (5)	71 (6)	65 (3)	62 (8)	54 (4)	49 (9)	40 (0)	15 (0)
Combo	108 (3)	105 (3)	102 (6)	96 (3)	93 (9)	84 (5)	79 (7)	72 (8)	64 (6)	58 (4)	54 (5)	48 (3)	45 (1)	44 (0)	19 (0)

Proportion of patients to achieve SARS-CoV-2 clearance over time. SARS-CoV-2 clearance (yes/no) is defined as 2 consecutive negative tests for the SARS-CoV-2 virus vs otherwise. The date of viral clearance is defined as the earliest date of the 2 consecutive negative tests. *a HR - stratified by duration since symptom onset to randomization category (<=8 days vs >8 days). *b Stratified log-rank for comparison with placebo. CI, confidence interval; HR, hazard ratio.

eFigure 2. Time to symptom improvement



No. of Patients at Risk

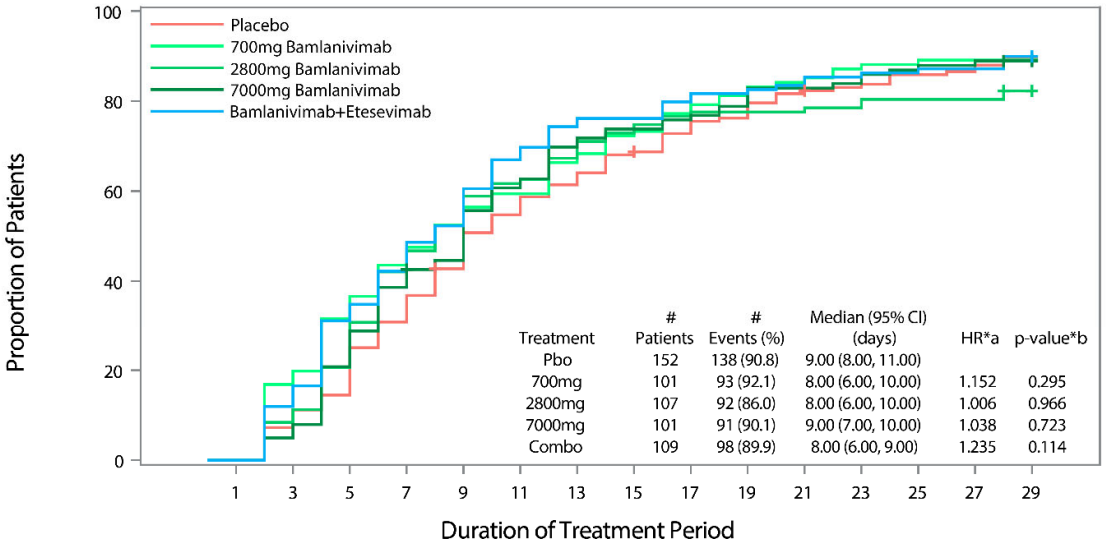
Time	1	3	5	7	9	11	13	15	17	19	21	23	25	27	29
Pbo	152 (24)	128 (29)	99 (18)	81 (23)	57 (11)	46 (9)	37 (5)	31 (9)	22 (6)	16 (2)	14 (2)	12 (2)	10 (1)	9 (1)	5 (2)
700mg	101 (25)	76 (23)	53 (15)	38 (9)	29 (3)	26 (7)	19 (5)	14 (3)	11 (1)	10 (1)	9 (1)	8 (0)	8 (0)	8 (0)	4 (1)
2800mg	107 (23)	84 (24)	60 (17)	43 (12)	31 (4)	27 (5)	22 (2)	20 (3)	17 (1)	16 (4)	12 (0)	12 (0)	12 (0)	12 (2)	2 (1)
7000mg	101 (14)	87 (30)	57 (19)	38 (11)	27 (5)	22 (5)	17 (2)	15 (3)	12 (5)	7 (0)	7 (0)	7 (0)	7 (0)	7 (0)	3 (1)
Combo	109 (26)	83 (28)	55 (15)	40 (10)	30 (7)	23 (5)	18 (0)	18 (1)	17 (1)	16 (3)	13 (0)	13 (1)	12 (0)	12 (3)	2 (0)

Proportion of patients to achieve symptom improvement over time. Symptom Improvement (yes/no) is defined as (1) all symptoms on the symptom questionnaire scored as moderate or severe at baseline are subsequently scored as mild or absent, AND (2) all symptoms on the symptom questionnaire scored as mild or absent at baseline are subsequently scored as absent vs otherwise.

*a HR - stratified by duration since symptom onset to randomization category (<=8 days vs >8 days).

*b Stratified log-rank for comparison with placebo. CI, confidence interval; HR, hazard ratio.

eFigure 3. Time to symptom resolution



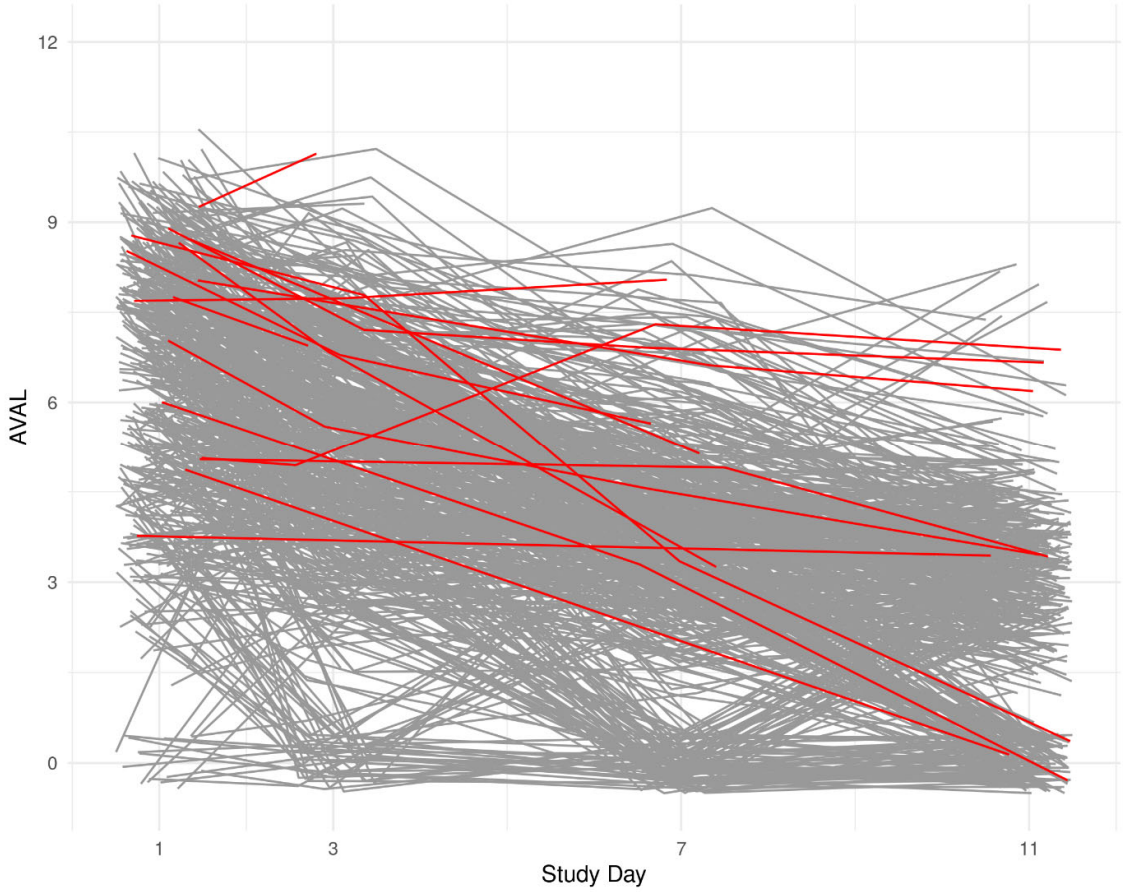
Treatment	# Patients	# Events (%)	Median (95% CI) (days)	HR*a	p-value*b
Pbo	152	138 (90.8)	9.00 (8.00, 11.00)		
700mg	101	93 (92.1)	8.00 (6.00, 10.00)	1.152	0.295
2800mg	107	92 (86.0)	8.00 (6.00, 10.00)	1.006	0.966
7000mg	101	91 (90.1)	9.00 (7.00, 10.00)	1.038	0.723
Combo	109	98 (89.9)	8.00 (6.00, 9.00)	1.235	0.114

No. of Patients at Risk

Time	1	3	5	7	9	11	13	15	17	19	21	23	25	27	29
Pbo	152 (17)	135 (21)	114 (18)	96 (21)	74 (12)	62 (8)	54 (7)	46 (10)	36 (6)	30 (4)	25 (2)	23 (3)	20 (3)	17 (2)	8 (4)
700mg	101 (20)	81 (17)	64 (11)	53 (9)	44 (3)	41 (9)	32 (5)	27 (6)	21 (4)	17 (2)	15 (3)	12 (1)	11 (0)	11 (0)	5 (3)
2800mg	107 (12)	95 (21)	74 (17)	57 (13)	44 (4)	40 (9)	31 (4)	27 (3)	24 (0)	24 (1)	23 (2)	21 (0)	21 (0)	21 (2)	8 (4)
7000mg	101 (8)	93 (21)	72 (14)	57 (13)	44 (7)	37 (9)	28 (2)	26 (3)	23 (6)	17 (0)	17 (3)	14 (2)	12 (1)	11 (0)	5 (2)
Combo	109 (18)	91 (20)	71 (15)	56 (13)	43 (10)	33 (7)	26 (0)	26 (6)	20 (1)	19 (3)	16 (1)	15 (1)	14 (0)	14 (3)	3 (0)

Proportion of patients to achieve symptom resolution over time. Symptom Resolution (yes/no) is defined as defined as all symptoms (excluding the loss of appetite and changes in taste and smell symptoms) on the symptom questionnaire scored as absent vs otherwise. HR>1 favors active treatment. *a HR - stratified by duration since symptom onset to randomization category (<=8 days vs >8 days). *b Stratified log-rank for comparison with placebo. CI, confidence interval; HR, hazard ratio

eFigure 4. Viral load over time for all patients



Viral load over time for all patients in this analysis (placebo, monotherapy, and combination). Log (viral load) calculated from N1/N2 cycle threshold value after normalization by RNase P(RP) CT. Red lines show patients who eventually reached the endpoint of ER visit or hospitalization and who had viral load data available. AVAL = Log_{10} (Viral Load).

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